

CLAIMS

1. Antibiotic 107891 complex comprising Factor A1 and Factor A2 being a white powder having the following characteristics:

(A) Mass spectrum recorded from a 0.2 mg/ml solution in
5 methanol:water 80/20 (v/v) with trifluoroacetic acid 0,1% (Fig. 1A and 1B) on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 220° C; capillary voltage: 3V;
10 infusion mode 10 µl/min, showing two double protonated ions at m/z 1124 and m/z 1116, corresponding to the lowest isotope composition of Factor A1 and A2, respectively.

(B) Infrared spectrum (Fig. 2) recorded in KBr with a Bruker FT-IR spectrophotometer model IFS 48, exhibiting absorption
15 maxima at (cm⁻¹): 3263; 2929; 1661; 1533; 1402; 1114; 1026.

(C) U.V. spectrum (Fig. 3), performed in methanol:H₂O 80:20 (v/v) with a Perkin-Elmer spectrophotometer Lambda 16, exhibiting two shoulders at 226 and 267 nm.

(D) ¹H-NMR spectrum (Fig. 4) recorded at 600 MHz in the
20 mixture methanol-d₄:H₂O (pH 4.3 HCl) 40:10 (v/v) at 40°C on a Bruker AMX 600 spectrometer applying a water suppression sequence using as internal standard the residual signal of methanol-d₄ at 3.31 ppm, exhibiting the following signals [δ=ppm multiplicity; (attribution)]: 0,93 d (CH₃), 0.98 d (CH₃),
25 1.07 t (overlapped CH₃'s), 1.18 t (overlapped CH₃'s), 1.26 s (CH₃), 1.30 t (overlapped CH₃'s), 1.62-1.74 m (CH₂), 1.78 d (CH₃), 1.80 d (CH₃), 2.03 m (CH₂), 2.24 m (CH), 2.36 m (CH₂), 2.72-3.8 m (peptidic alpha CH's), 3.8-5.2 m (peptidic alpha CH's), 5.53-6.08 s (CH₂), 5.62 d (CH double bond), 6.42 m (CH),
30 6.92 d (CH double bond), 7.0-7.55 m (aromatic CH's), 7.62-10.4 d and m (aromatic and peptidic NH's).

(E) ¹³C-NMR spectrum (Fig. 5) recorded in the mixture methanol-d₄:H₂O (pH 4.3 HCl) 40:10 (v/v) at 40°C on a Bruker AMX 600 spectrometer, using as internal standard the residual
35 signal of methanol-d₄ at 49.15 ppm, exhibiting the following

signals: [δ =ppm; (attribution)]: 13:6-23.2 (aliphatic CH₃'s), 26.16-73 (aliphatic CH₂'s and peptidic alpha CH's), 105-136 (aromatic and double bonds CH's and quaternary carbons), 164.3-176.3 (peptidic carbonyls).

5 (F) The acid hydrolysate in 6N HCl, (105°C, 24 h) showing the presence of the following amino acids, along with other unidentified peaks, after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate: lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid
10 (hydrolysis product of asparagine), phenylalanine and leucine.

G) The acid hydrolysate in 4N methanesulfonic acid containing 0,2% (w/v) 3-(2-aminoethyl) indole as catalyst (115°C, 16h) showing the presence of 5-chlorotryptophan.

H) A basic ionizable function detected by acid/base
15 titration performed with 0.01 N potassium hydroxide in 2-methoxyethanol (MCS):H₂O 12:3 (v/v) containing a molar excess of 0.01 N hydrochloric acid.

2) Factor A1 of antibiotic 107891 being a white powder showing:

A) A doubly protonated ion at m/z 1124 corresponding to the
20 lowest isotope composition in mass spectrum recorded from a 0,1 mg/ml solution in acetonitrile:water 50:50 (v/v) with acetic acid 0,5% (Fig. 6A and 6B) on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix under the following
25 electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 250° C; capillary voltage: 8V; infusion mode 10 μ l/min.

B) The exact mass of antibiotic determined by using a Bruker
Daltonics APEX II, 4.7 Tesla spectrometer fitted with an
30 electrospray source, corresponding to a molecular weight of 2246.71 \pm 0.06, calculated monoisotopic mass from [M+2H]²⁺ at m/z 1124.36124 (accuracy 30 ppm).

C) When dissolved in CD₃CN:D₂O (1:1), ¹H NMR spectrum (Fig.8) exhibiting the following groups of signals (in ppm) at 600

MHz using CD_3CN as internal standard (1.94 ppm), [δ =ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.89 d (CH_3), 0.94 t (overlapped CH_3 's), 1.1 d (CH_3), 1.13 d (CH_3), 1.15 t (overlapped CH_3 's), 1.49 m' (CH_2), 1.69 d (CH_3), 1.75 m (CH_2), 2.11 m (CH), 2.26 m (CH), 2.5 m (CH_2), 2.68 - 3.8 m (peptidic CH_β 's), 3.8 - 5.0 m (peptidic CH_α 's), 5.45 - 6.17 s (CH_2), 5.58 d (CH double bond), 6.36 m (CH), 6.86 d (CH double bond), 7.0 - 7.45 m aromatic CH's).

10 D) When dissolved in $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ (1:1), ^{13}C NMR spectrum (Fig.10) exhibiting the following signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.39 ppm), [δ =ppm; (attribution)]: 13.6 - 23.03 (aliphatic CH_3 's), 25.69 - 77.9 (aliphatic CH_2 's and peptidic CH_α 's), 105 - 137.3 (aromatic and double bonds
15 CH's and quaternary carbons), 165.6- 176.6 (peptidic carbonyls).

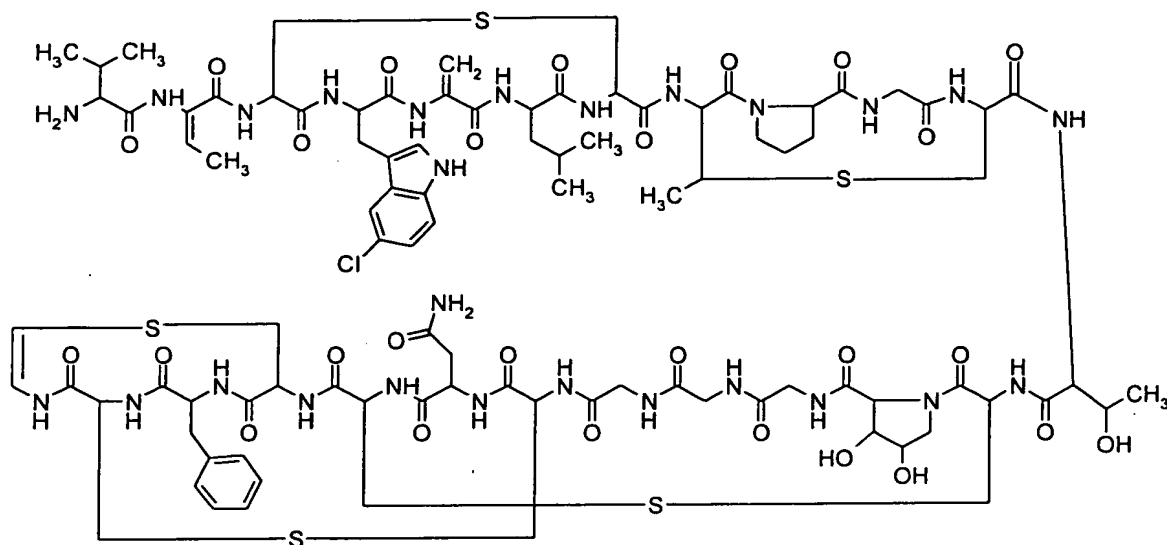
E) Infrared spectrum recorded in KBr with a Bruker FT-IR spectrophotometer model IFS 48 (Fig. 12) exhibiting absorption maxima at (cm^{-1}): 3294; 3059; 2926; 1661; 1529; 1433; 1407;
20 1287; 1114; 1021.

F) U.V. spectrum recorded in methanol: H_2O (in ratio 80:20) with a Perkin-Elmer spectrophotometer Lambda 16 (Fig. 13) exhibiting two shoulders at 226 and 267 nm.

G) The acid hydrolysate in 6N HCl, (105°C , 24 h) showing the
25 presence of the following amino acids, along with other unidentified peaks, after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate: lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (hydrolysis product of asparagine), phenylalanine, and
30 leucine.

H) The acid hydrolysate in 4N methanesulfonic acid containing 0,2% (w/v) 3-(2-aminoethyl)indole as catalyst (115°C , 16h) showing the presence of 5-chlorotryptophan.

3. Antibiotic 107891 Factor A1 according to claim 2 which can
35 be tentatively assigned the following structure formula:



4. Factor A2 of antibiotic 107891 being a white powder showing:

- 5 A) A doubly protonated ion at m/z 1116 corresponding to the lowest isotope composition in mass spectrum recorded from a 0,1 mg/ml solution in acetonitrile:water 50:50 (v/v) with acetic acid 0,5% (Fig. 7A and 7B) on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using
10 Thermofinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 250° C; capillary voltage: 8V; infusion mode 10 μ l/min.
- B) The exact mass determined by using a Bruker Daltonics APEX
15 II, 4.7 Tesla spectrometer fitted with an electrospray source, corresponding to a molecular weight of 2230.71 ± 0.06 , calculated monoisotopic mass from $[M+2H]^{2+}$ at m/z 1116.36260 (accuracy 30 ppm).
- C) When dissolved in $CD_3CN:D_2O$ (1:1), 1H NMR spectrum (Fig. 9)
20 exhibiting the following signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.94 ppm), [δ =ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.88 d (CH_3), 0.94 d

(CH₃), 1.06 d (CH₃), 1.14 d (CH₃), 1.48 m (CH₂), 1.65-1.75 m (CH₂), 1.67 d (CH₃), 2.15 m (CH), 2.25 m (CH), 2.5 m (CH₂), 2.77 - 3.8 m (peptidic CH_β's), 3.8 - 4.9 m (peptidic CH_α's), 5.45 - 6.14 s (CH₂), 5.59 d (CH double bond), 6.34 m (CH), 6.84 d (CH double bond), 7.0 - 7.42 m (aromatic CH's).

D) When dissolved in CD₃CN:D₂O (1:1), ¹³C NMR spectrum (Fig.11), exhibiting the following signals (in ppm) at 600 MHz using CD₃CN as internal standard (1.39 ppm), [δ=ppm; (attribution)]: 13.6 - 22.9 (aliphatic CH₃'s), 25.65 - 73 (aliphatic CH₂'s and peptidic CH_α's), 105 - 137.3 (aromatic and double bonds CH's and quaternary carbons), 165.7- 176.1 (peptidic carbonyls).

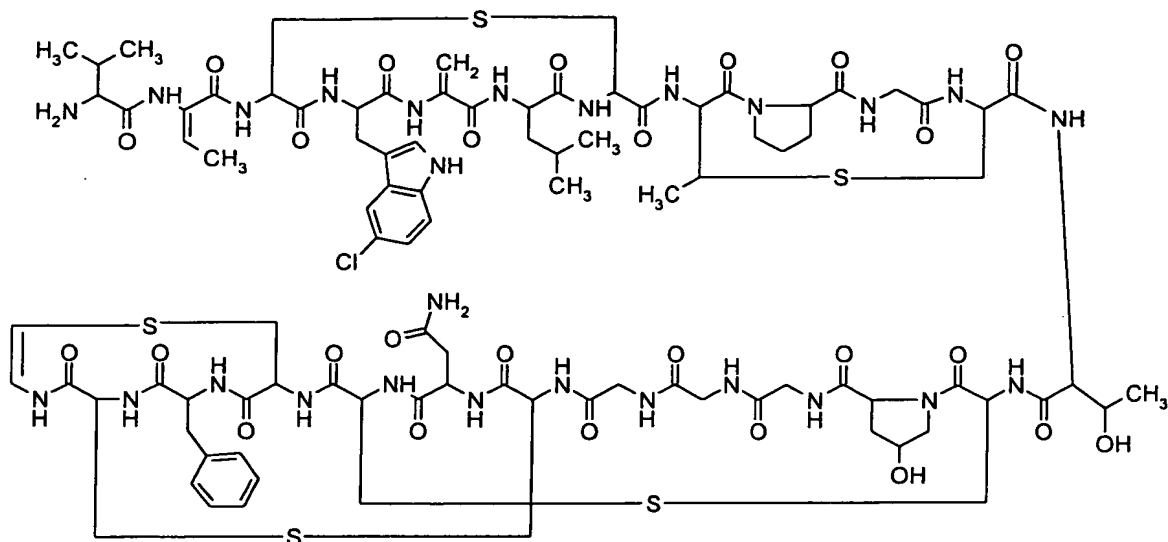
E) Infrared spectrum recorded in KBr with a Bruker FT-IR spectrophotometer model IFS 48 (Fig. 14), exhibiting absorption maxima at (cm⁻¹): 3296; 3060; 2928; 1661; 1529; 1433; 1407; 1288; 1116.

F) U.V. spectrum recorded in methanol:H₂O (in ratio 80:20) with a Perkin-Elmer spectrophotometer Lambda 16 (Fig. 15) exhibiting two shoulders at 226 and 267 nm.

G) The acid hydrolysate in 6N HCl, (105°C, 24 h) showing the presence of the following amino acids, along with other unidentified peaks, after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate: lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (hydrolysis product of asparagine), phenylalanine and leucine.

H) The acid hydrolysate in 4N methanesulfonic acid containing 0,2% (w/v) 3-(2-aminoethyl)indole as catalyst (115°C, 16h) showing the presence 5-chlorotryptophan.

5. Antibiotic 107891 Factor A2 according to claim 4 which can be tentatively assigned the following structure formula



6. A process for producing antibiotic 107891 and its Factors A1 and A2 and the salts thereof with acids as defined in claim 1 which comprises :

- 5 - cultivating *Microbispora* sp. ATCC PTA-5024 or a variant or mutant thereof maintaining the ability to produce said antibiotic, under aerobic conditions, in an aqueous nutrient medium containing an assimilable source of carbon, nitrogen and inorganic salts;
- 10 - isolating the resulting antibiotic from the mycelium and/or the filtered fermentation broth;
- purifying the isolated antibiotic 107891 and, optionally, separating Factor A1 and Factor A2 therefrom.

7. A process according to claim 6, wherein the strain
15 *Microbispora* sp. ATCC PTA-5024 or the antibiotic 107891 producing a variant or mutant thereof are pre-cultured.

8. A process according to any of claims 6 and 7, wherein the
isolation of the antibiotic 107891 is carried out by filtering
the fermentation broth and the antibiotic is recovered from
20 the filtered fermentation broth according to a technique
selected from: extraction with a water-immiscible solvent,
precipitation by adding a non-solvent or by changing the pH of
the solution, absorption chromatography, partition

chromatography, reverse phase partition chromatography, ion exchange chromatography, molecular exclusion chromatography, and a combination of two or more of said techniques.

9. A process according to any of claims 6 and 7, wherein the isolation of the antibiotic 107891 is carried out by separating the mycelium from the supernatant of the fermentation broth and the mycelium is extracted with a water-miscible solvent whereby, after the removal of the spent mycelium, a water-miscible solution containing the crude antibiotic is obtained, which can be processed either separately or in pool with the filtered fermentation broth according to claim 8 to recover the antibiotic 107891 by means of a technique selected from: extraction with a solvent, precipitation by adding a non-solvent or by changing the pH of the solution, absorption chromatography, partition chromatography, reverse phase partition chromatography, ion exchange chromatography and molecular exclusion chromatography, or a combination of two or more of said techniques.

10. A process as in claim 9 whereby the concentration of the water-miscible solvent in the mycelium extract is reduced before it is processed to recover the antibiotic therefrom.

11. A process according to claim 8 whereby the filtered fermentation broth is contacted with an absorption resin, preferably a polystyrene, a mixed polystyrene-divinylbenzene or a polyamide resin, and said resin is eluted with a polar, water-miscible solvent or a mixture thereof with water, whereby a solution containing the crude antibiotic 107981 is obtained.

12. A process as in any of claims 9 and 10 wherein the mycelium is extracted with a C₁-C₃ alkanol, preferably methanol, and the mycelium extract is contacted with an absorption resin, preferably a polystyrene resin, and eluted therefrom with a polar water-miscible solvent or a mixture thereof with water, whereby a solution containing the crude antibiotic 107891 is obtained.

13. A process as in any of claims 8, 9, 10 and 12, wherein the solutions containing the crude antibiotic 107891 are pooled and processed for further purification of said antibiotic 107891.
- 5 14. A process as in any of claims 11, 12 and 13, wherein the solution containing the crude antibiotic 107981 is concentrated and then freeze-dried to yield a crude antibiotic 107891 solid product.
- 10 15. A process as in any of claims 11 and 12, wherein the absorption resins containing the absorbed antibiotic are pooled and their mixture is eluted with a polar, water-miscible solvent or a mixture thereof with water.
- 15 16. A process according to any of claims 6 to 15 wherein the antibiotic 107981 is purified by means of a chromatographic procedure, preferably by preparative HPLC or medium pressure chromatography.
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17. A process according to any claims 6 to 16, wherein Factor A1 and Factor A2 are separated by preparative HPLC from the purified antibiotic 107891.
- 20 18. A pharmaceutical composition comprising an antibiotic selected from antibiotic 107891, its Factor A1, its Factor A2 according to any of claims 1 to 5 and a mixture of said Factors in any proportion or a pharmaceutically acceptable salt thereof with an acid.
- 25 19. A pharmaceutical composition according to claim 18, comprising a pharmaceutically acceptable carrier.
20. The antibiotic 107891, its Factor A1, its Factor A2, according to any of claims 1 to 5 or a mixture of said Factors in any proportion or a pharmaceutically acceptable salt thereof with an acid for use as a medicament.
- 30 21. Use of antibiotic 107891, its Factor A1, its Factor A2, according to any of claims 1 to 5, or a mixture of said Factors in any proportion or a pharmaceutically acceptable salt thereof with an acid for the manufacture of a medicament
- 35 for the treatment or prevention of bacterial infections.

22. Use of the antibiotic 107891, its Factor A1, its Factor A2 according to any of claims 1 to 5 or a mixture of said Factors in any proportion and a non-toxic salt thereof with an acid as animal growth promoter.

5 23. A biologically pure culture of the strain *Microbispora* sp. ATCC PTA-5024, or a variant or mutant thereof maintaining the ability to produce the antibiotic of claim 1 when cultivated under submerged aerobic conditions in the presence of assimilable sources of carbon, nitrogen and inorganic salts.